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IN THE UNITED STATES PATENT OFFICE

Application Serial No. 07/675,908

Filed: July 3, 1991

Applicants: Dr. Rudolf Falk
Dr. Samuel S. Asculai
(Now assigned to Hyal Pharmaceutical Corporation)

Title: THE USE OF HYALURONIC ACID OR ITS DERIVATIVES TO ENHANCE DELIVERY OF ANTINEOPLASTIC AGENTS

Inventors: Dr. Rudolf Falk,
Dr. Samuel S. Asculai

Examiner: Dr. Jacqueline Krikorian Ph.D. (*formerly* Dr. Stephen Martin, Ph.D.)

Group Art Unit: 1806 Extended Due Date: September 5, 1996

The Commissioner of Patents
UNITED STATES PATENT OFFICE
2011 Jefferson Davis Highway
Crystal Plaza 2, Room 1B03
Arlington, Virginia
U.S.A. 22202

DECLARATION OF TORVARD C. LAURENT
under § 1.132

I, TORVARD C. LAURENT, make oath and say as follows:

1. I am a Professor emeritus in Medical and Physiological Chemistry and have been same from 1966 to 1996. A brief copy of my Curriculum Vitae is attached as ***Exhibit 1*** to this my Declaration.

2. I am both a Doctor of Medicine and a Doctor of Medical Science having obtained both degrees in 1958. I have published over 200 papers primarily on:

(i) chemistry of connective tissue (especially the physical properties), physiological functions, turnover and medical applications of the polysaccharide hyaluronan (hyaluronic acid),

(ii) ophthalmic biochemistry,

(iii) physical chemistry of polysaccharide networks (especially transport processes), and polysaccharide solutions, and

(iv) biochemical separation techniques, e.g. a theory of gel filtration (1964) and methods for cell separation.

In fact, I became knowledgeable, very early on in my profession, with the nature of hyaluronic acid and have written a substantial number of papers in respect thereto. I have identified those publications in my listing of publications which is attached as *Exhibit* 2 to this my Declaration which relate to hyaluronic acid.

3. I have been asked for my opinion in respect of International Application No. PCT/CA 90/00306 published under International Publication No. WO 91/04058 which, I am advised by Ivor Hughes Counsel for Hyal Pharmaceutical Corporation, entered the National Phase in the United States as U.S. Patent Application Serial No. 07/675,908, inventors, Drs. Falk and Asculai.

4. The inventors have found that when some traditional drugs are mixed with the polysaccharide hyaluronan (hyaluronic acid) and administered to patients, the polysaccharide potentiates the action of the drugs. The effect has mainly been demonstrated on a number of patients with incurable cancer.

5. When I first was informed of this effect, I was surprised for two reasons. The inventors had injected intravenously very high doses of hyaluronan (in

some cases several grams per day) without any adverse effects on the patients. This was contrary to what was believed to be possible as the maximal uptake of hyaluronan from blood had been estimated to be 350 mg. per day. Secondly, I was not aware of any known mechanism by which high-molecular weight hyaluronan could potentiate the effects of drugs. We still do not know what the mechanism could be but in recent years we have learnt much more about the specific interactions between hyaluronan and cells which at least gives a basis for speculation.

6. To my knowledge it has not previously been claimed that hyaluronan potentiates the biological effect of a drug. In a case when hyaluronan was mixed with a drug e.g. for topical application (see e.g. U.S. Patent 4,736,024) hyaluronan was used as a vehicle for the drug but not assumed to have any effect on the tissue itself. In other patents I have read (Schultz et al, U.S. Patent 4,808,576; Fidia SpA, European Patent Application 0138572 A2) it has been claimed that hyaluronan when administered *alone* has a pharmaceutical activity.

7. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements will jeopardize the validity of the application and any patent issuing thereon.

EXECUTED this 26 day of August, 1996.

TORVARD C. LAURENT

EXHIBIT 1

CURRICULUM VITAE

Name:	**Torvard C Laurent**
Profession:	Professor emeritus in Medical and Physiological Chemistry
Born:	December 5, 1930 in Stockholm, Sweden
Nationality:	Swedish
Family status:	Married to Ulla B.G. Laurent b. Hellsing, MD, Ophthalmologist Children: Birgitta b. 1957, Claes b. 1958, Agneta b. 1960.
Address:	Home: Hävelvägen 9, S-756 47 Uppsala, Sweden. Phone: Intern.+46-(0)18-309612. Work: Department of Medical and Physiological Chemistry, University of Uppsala, Box 575, S-75123, Uppsala Sweden. Phone: Intern+46-(0)18-174155. Fax: Intern.+46-(0)18-174975. Wenner-Gren Center, Sveavägen 166, 23rd floor, S-113 46 Stockholm, Sweden. Phone: Intern.+46-(0)8-7369816. Fax: Intern.+46-(0)8-318632
Education:	Karolinska Institute, Stockholm: Bachelor of Medicine 1950 Doctor of Medicine 1958 Doctor of Medical Science (research degree) 1958.
Positions:	Instructor and teacher in Histology and Chemistry at Karolinska Institutet 1949-52 and 1955-58 Research fellow and research associate at Retina Foundation, Boston, MA, USA 1953-54 and 1959-61 Established Investigator in Ophthalmic Chemistry at the Swedish Medical Research Council and Associate Professor at the University of Uppsala 1961-66 Professor of Medical and Physiological Chemistry, University of Uppsala 1966-96 Visiting Professor in Biochemistry at Monash University, Australia 1979-80

Career-related activities (selected):

Swedish Biochemical Society	
Secretary	1967-70
Chairman	1973-76
Member of the Swedish Natural Science Research Council	1968-70
Member of the Swedish Medical Research Council	1970-77
Deputy Chairman	1973-77
Chairman of the Dept.of Medical and Physiological Chemistry	1973-77 1987-91
Deputy Chairman Biomedical Center in Uppsala	1987-91
Deputy dean, Faculty of Medicine Univ.of Uppsala	1969-72
Member of the Board of the University of Uppsala	1988-91
Board of the Sätra Spa (owned by UU)	
Member	1972-91
Chairman	1980-91
Gustaf V's 80 year Birthday Fund	
Member of the scientific board	1970-96
Member of the council	1987-93
Member of the board	1993-
Royal Swedish Academy of Sciences	
Member	1982-
President	1991-94
Member Nobel Committee for Chemistry	1992-
Council of the Nobel Foundation	
Member	1992-
Chairman	1994-
Academia Europea (London)(member)	1991-
Academia Scientarum et Artium Europea (Salzburg)(member)	1991-
Member of the board and scientific secretary of the Wenner-Gren Foundation,	1993-
Member of the Standing Finance Committee of the International Council of Scientific Unions in Paris	1994-
Auditor of the Fund for Strategic Research, Stockholm	1995-
Member of the board of Medisan Ltd	1994-

Awards and honors:

The prize of King Oscar II from the University of Uppsala	1965
Anders Jahre's medical prize for young scientists from University of Oslo	1968
Pharmacia award, Pharmacia Inc, Uppsala	1986
Eric K. Fernström Medical Prize from University of Lund	1989
Björken's prize from the University of Uppsala	1990
The Hedlinger Gold Medal from the University of Uppsala	1991
Doctor of Medicine honoris causa, University of Turku, Finland	1993
Doctor of Chemistry and Pharmaceutical Technology honoris causa, University of Bologna, Italy	1994

King Carl XVI Gustaf´s Goldmedal 1994

Creative work & achievements: Published about 210 papers mainly on (i) chemistry of connective tissue, especially the physical properties, physiological functions, turnover and medical applications of the polysaccharide hyaluronan (hyaluronic acid); (ii) ophthalmic biochemistry; (iii) physical chemistry of polysaccharide networks, especially transport processes in polysaccharide solutions; and (iv) biochemical separation techniques e.g. a theory of gel filtration (1964) and methods for cell separation.

Curriculum vitae

TORVARD C. LAURENT (born December 5,1930).

EXAMS

Student vid Lidingö hal	1948
Medicine kandidat, Karolinska Institutet	1950
Medicine licentiat, Karolinska Institutet	1958
Disputation för medicine doktorsgrad vid Karolinska institutet	1957
Docent i medicinsk fysikalisk kemi vid Karolinska institutet	1957-1962
Docent i medicinsk kemi, Uppsala Universitet	1962-1966

APPOINTMENTS

Amanuens i histologi, Karolinska institutet	1949-1951
Amanuens i kemi, Karolinska institutet	1951-1952
Research fellow, Retina Foundation, Boston MA Fellowship från National Council to Combat Blindness, New York, NY	1953-1954
Assistent och biträdande lärare i kemi, Karolinska institutet	1955-1958
Research Associate, Retina Foundation. Fellowship från Helen Hay Whitney Foundation, New York, NY	1959-1961
Forskartjänst i ögats biokemi vid Medicinska Forskningsrådet med placering i Uppsala	1961-1963
Forskardocent, Uppsala Universitet	1964-1966
Professor i medicinsk och fysiologisk kemi, Uppsala Universitet	1966-1996
Visiting Professor in Biochemistry, Monash University, Melbourne, Australia	1979-1980

TEACHING

Undervisat i allmän kemi, framför allt fysikalisk kemi, vid Karolinska Institutet under 1950-talet.

Undervisat i medicinsk och fysiologisk kemi i läkarutbildningen som professor i ämnet i Uppsala.

Organiserat och/eller föreläst i en rad forskarutbildningskurser inom och utom landet främst i biofysikalisk kemi, ögonbiokemi, bindvävsbiokemi och reumatologi. Tillsammans med Charles Kurland initiativtagarna till BMC-Summer Schools. Initiativtagare till de årliga forskarutbildningskurserna på Sätra Brunn.

AWARDS

Oscar II:s jubileumspris från Uppsala Universitet 1965.
Anders Jahres medicinska pris till yngre forskare från

Oslo Universitet 1968.
Pharmacia Award, 1986.
Eric K. Fernströms Stora Nordiska Pris i Medicin från Lunds Universitet 1989.
Björkénska priset från Uppsala Universitet 1990
Den äldre Gustaf Adolfs medaljen i guld från Uppsala Universitet 1992.
Ett laboratorium vid Pharmacia döpt till Torvard Laurent-laboratoriet. 1993
Medicine hedersdoktorat i Åbo 1993
Hedersdoktor i farmaceutisk kemi och teknologi, Bologna 1994.
Kung Carl XVI Gustafs medalj i 12 storleken 1994

ADMINISTRATION

Vid Uppsala Universitet	
Ledamot av medicinska fakultetsnämnden	1967-1972
Prodekanus	1969-1972
Suppleant för dekanus i fakultetsnämnden	1987-1991
Ordförande i medicinska fakultetens forskarutbildningsnämnd	1969-1973 1980-1983
Ledamot i samma nämnd	1983-1987
Ordförande i medicinska fakultetens docenturnämnd	1973-1976
Ledamot av fakultetens tjänsteberednings-nämnd	1984-1991
Ledamot av Akademiska Sjukhusets direktion	1970-1976
Ledamot av Sätra Brunns styrelse	1972-1991
varav som ordförande	1980-1991
Ledamot och vice ordförande i BMC-nämnden	1973-1977 1987-1991
Ställföreträdande prefekt för BMC	1987-1991
Inspektor för Lennanderstipendierna	1973-1983
UU:s representant i veterinärlinjenämnden	1977-1983
Prefekt vid institutionen för Medicinsk och Fysiologisk kemi.	1973-1977 1987-1991
Ledamot av Uppsala universitets styrelse	1988-1991
Vid forskningsråd och fonder.	
Ledamot av Statens Naturvetenskapliga Forskningsråd	1968-1970
Ledamot i kemidelegationen	1968-1970
Ledamot i biologidelegationen	1969-1970
Ledamot av Statens Medicinska Forsknings-råd	1970-1977
Vice ordförande tillika ordförande i arbetsutskottet	1973-1977
Ordförande i prioriteringsgrupp Kemi II	1970-1977
Ledamot av forskningsnämnden för Gustaf V:s 80-årsfond	1970-1996
Huvudman för samma fond	1987-1993
Styrelseledamot samma fond	1993-
Ledamot i Nationalkommittén för Biokemi	1982-1988

Ordförande	1985-1988
Ledamot och sekreterare i Kommittén för The Svedberg-stipendierna	1982-1988
Medlem i styrgruppen för Pharmacias anslag till Akademiska Sjukhuset	1984-1990
Ledamot i Nobelkommittén förkemi adjung.	1991-1995
Ordinarie	1996-
Ledamot av Nobelfullmäktige	1992-
Ordförande	1994-
Vetenskaplig sekreterare i Wenner-Gren stiftelserna	1993-
Revisor i Fonden för Strategisk Forskning	1995-
Ledamot av ICSUs finanskommitté	1995-

I näringslivet.

Ledamot av "academic council" av World Economic Forum	1991-
Scientific advisory council Biomatrix Inc	1991-
Scientific advisory council of HYAL Inc	1994-
Styrelseledamot och vetenskapligt råd i Medisan	1994-

I vetenskapliga samfund.

Experimentalbiologiska sällskapet i Uppsala	
Sekreterare	1963-1965
Ordförande	1965-1966
Sveriges Biokemiska Förening, sekreterare	1967-1970
Ordförande	1973-1976
Kungliga Vetenskapssamhället i Uppsala (invald)	1969
Vice preses	1980-1983
Kungliga Vetenskapssocieten (invald)	1974
Vice sekreterare	1982-
Ledamot av Kungliga Vetenskapsakademiens VIII klass (invald)	1982
Preses	1991-
Academia Europea (invald)	1991
Academia Scientiarum et Artium Europaea (invald)	1991

Organisation av konferenser.

Ledamot av organisationskommittén för symposiet "Biology of Fibroblast" i Åbo	1972
Ledamot av programkommittén vid Internationella Biokemikongressen i Stockholm och arrangör av kollokviet "Biochemistry of Extracellular Matrix"	1973
Arrangör av internationella symposiet "Biology of Connective Tissue" i Uppsala	1977
Ledamot av organisationskommittén for European Science Foundations workshop "Specific Interaction in Polysaccharide Systems" i Uppsala	1983
Ledamot av Scientific Committee för "7th Inter-	

national Symposium on Glycoconjugates" i Ronneby 1983
Medarrangör av symposiet "Biological Activity and Metabolism of Hyaluronan" i St.Tropez 1985
Medlem av organisationskommittén för symposiet "The Clinical Impact of Bone and Connective Tissue Markers" i Uppsala 1988
Initiativtagare och ordförande vid CIBA-symposiet "The Biology of Hyaluronan" i London 1988
Organisatör av post-graduatekursen "Biology of Hyaluronan" på Sätra Brunn 1991
Organiserat och ordförande vid symposiet "The Biological Role of Hyaluronan with Special Reference to the Normal and Pathological Eye." Johannesberg, Uppsala 1993
Organiserat mötet "Matrix Biology" Gemensamt möte mellan de svenska och australiska vetenskapakademierna i Medevi 1993
Organiserat kontaktkonferens mellan BMC och Kabi Pharmacia i Johannesbergs slott 1993
Värd vid Academia Europeas symposium på WGC "Goals and purposes of higher education in the 21st century" 1994
Organiserat symposiet "Glycosaminoglycans and connective tissue during the Rodénian era" i Birmingham AL 13-14 nov. 1994
Organiserat en diskussion på Wenner-Gren Center om samarbete mellan universitet och industri 1995
Organiserat WGC-symposiet "Politics and culture during the time of Queen Christina" på WGC 1995

Har dessutom arrangerat ett flertal möten med Sveriges Biokemiska Förening, MFR:s planeringsgrupp "Bindvävens biologi", Svenska Bindvävsklubben liksom sessioner inom ramen för nationella och internationella medicinska och biokemiska konferenser.

GRADUATE STUDENTS
Dissertations in in the research gropup (pupils(*) or pupil's pupils).

* Arvid Anseth, 1961. "Glycosaminoglycans in the corneal stroma and their alterations during development and regeneration." Arbetet utfört i Boston. Disputation vid ögonkliniken i Lund.

* Ingemar Björk, 1964. "Studies on the soluble proteins of the bovine lens. Purification and characterization."

* Ulf Lindahl, 1966. "Structure of the heparin-protein linkage region." Avhandlingen påbörjad i Chicago under ledning av Lennart Rodén.

* Krister Hellsing, 1968. "On the solubility of antigen-antibody complexes in the presence of dextran and some glycosaminoglycans from connective tissue."

* Torsten Helting, 1970. "Studies on the structure and biosynthesis of the protein-polysaccharide linkage regions of chondroitin sulfate and heparin."

* Håkan Pertoft, 1970. "Preparation of cells and viruses in colloidal silica gradients."

* Åke Wasteson, 1970. "A method for the determination of the molecular weight of chondroitins sulphate and its application to studies of the structure and metabolism of connective tissue polysaccharides."

* Björn Öbrink, 1971. "Studies on the interactions between collagen and glycosaminoglycans."

* Per-Henrik Iverius, 1971. "Polysaccharide interactions with plasma lipoproteins and lipoprotein lipase."

* Anund Hallén, 1974. "Application of ion exchange chromatography to the study of connective tissue glycosaminoglycans."

Magnus Höök, 1974. "Studies on the biosynthesis of heparin-like glycosaminoglycans." Handledare: Ulf Lindahl.

Leif Jansson, 1974. "Studies on the macromolecular properties and metabolism of heparin-like glycosaminoglycans." Handledare: Ulf Lindahl.

Sören Ögren, 1974. "Degradation of macromolecular heparin in mammalian tissues." Handledare: Ulf Lindahl.

Erik Lindh, 1975. "Studies on human secretory immunoglobulins. Gross conformation of secretory IgA and binding of secretory component in immunoglobulins A and M. Handledare: Ingemar Björk.

Ann-Margret Östlund, 1978. "Lipoproteinlipase and salt resistant lipase. Purification, characterization and studies on the activation of lipases from bovine milk and human post-heparin plasma." Handledare: Per-Henrik Iverius.

* Ove Wik, 1979. "Physico-chemical studies on hyaluronate."

Kristofer Rubin, 1980. "Interactions between rat hepatocytes, collagen and fibronectin in cell adhesion phenomena." Handledare: Björn Öbrink.

Carl-Henrik Heldin, 1980. "Studies on growth factors for human cultured cells." Handledare: Åke Wasteson.

Göran Åkerström, 1980. "Parenchymal cell mass determination in parathyroid diagnosis." Utgick från kirurgiska kliniken, UAS. Handledare på vår institution: Håkan Pertoft.

* Anders Tengblad, 1981. "Studies on the interaction between proteins and glycosaminoglycans. Some aspects on the interactions between heparin, antithrombin and thrombin; and hyaluronate, cartilage proteoglycan and cartilage link-protein."

* Inger Marie S Dahl, 1981."Biosynthesis of proteoglycans and hyaluronate in corneal tissue culture." Avhandlingen försvarades vid ögonkliniken, Tromsö Universitet.

Lena Kjellén, 1981. "Studies on rat liver heparan sulfate proteoglycans." Handledare: Magnus Höök.

* Ulla Laurent, 1982. "Studies on endogenous sodium hyaluronate in the eye." Försvarad vid ögonkliniken, UAS.

Staffan Johansson, 1983. "Pericellular matrix components and cell adhesion." Handledare: Magnus Höök.

Carin Ocklind, 1983. "Cell surface molecules involved in adhesion of rat hepatocytes." Handledare Björn Öbrink.

Börje Norling, 1983. "Proteoglycans of cultured cells. Structure, metabolism and organization in the extracellular matrix." Utgick från veterinärmedicinsk kemi. Handledare: Åke Wasteson.

* Monica Einarsson, 1983. "Studies on the hepatitis B virus. Isolation of hepatitis B surface antigen from human serum and removal of hepatitis B virus from blood products."

Claes Rudberg, 1984. "Pathophysiology and morphology of primary hyperparathyroidism." Utgick från kirurgiska kliniken, UAS. Handledare: Göran Åkerström och Håkan Pertoft.

* Bård Smedsröd, 1984. "Endocytosis of connective tissue macromolecules in liver endothelial cells." Utgick från veterinärmedicinsk kemi.

* Simon King, 1984. "Diffusion in microporous membranes." Avhandlingen försvarad vid Department of Biochemistry, Monash University, Australien.

IN THE UNITED STATES PATENT OFFICE

Application Serial No. 07/675,908

Filed: July 3, 1991

Applicants: Dr. Rudolf Falk
Dr. Samuel S. Asculai
(Now assigned to
Hyal Pharmaceutical Corporation)

Title: THE USE OF HYALURONIC ACID OR ITS DERIVATIVES TO ENHANCE DELIVERY OF ANTINEOPLASTIC AGENTS

Inventors: Dr. Rudolf Falk,
Dr. Samuel S. Asculai

Examiner: Dr. Jacqueline Krikorian Ph.D. (*formerly* Dr. Stephen Martin, Ph.D.)

Group Art Unit: 1806 Extended Due Date: September 5, 1996

The Commissioner of Patents
UNITED STATES PATENT OFFICE
2011 Jefferson Davis Highway
Crystal Plaza 2, Room 1B03
Arlington, Virginia
U.S.A. 22202

DECLARATION OF STEFAN GUSTAFSON
under § 1.132

I, STEFAN GUSTAFSON, make oath and say as follows:

1. I am a Master of Pharmacy and a Doctor of Medicine, the degrees of which I obtained in 1980 and 1986 respectively. Attached as *Exhibit 1* to this my Declaration is a copy of my Curriculum Vitae.

2. I have mainly been studying metabolism of lipoproteins and hyaluronan, especially receptor mediated endocytosis in reticuloendothelial cells (i.e. macrophages and liver endothelial cells). The work consists of purification and characterization of lipoproteins, polysaccharides and cell surface receptors for these ligands, together with binding, uptake and clearance studies *in vitro* and *in vivo*. I have also developed immunological assays for proteins in serum and lymph, and used these in clinically oriented projects. The majority of the work is carried out at the Department of Medical and Physiological Chemistry, Biomedical Centre, Uppsala University. I also have collaboration with Pharmacia and Upjohn, Departments of Surgery, Dermatology and Oncology at the University Hospital (primarily Wilhelm Graf, Lennart Juhlin, Jan-Erik Westlin and Hans Hagberg), the PET-centre at University of Uppsala, and other departments at the Biomedical Centre and the University Hospital. I supervise several graduate students (one became a doctor of Medical Science, May 1996) and medical students working primarily with hyaluronan receptor studies. I have also written extensively in respect of the above subject matter. I, therefore, consider myself an expert in respect of hyaluronan.

3. I have also assisted Hyal Pharmaceutical Corporation, the Assignee of this above-identified patent application in the U.S. Patent Office in other respects as a consultant. As a consultant, I have been involved in advising Hyal Pharmaceutical Corporation in all aspects of hyaluronan and have carried out research and development and testing for Hyal Pharmaceutical Corporation. I have also been identified as an inventor in an application relating to hyaluronan filed in the U.S. Patent Office and which has been assigned to Hyal Pharmaceutical Corporation. I would not, however, let my acting as a Consultant for Hyal Pharmaceutical Corporation interfere with my professional objectivity and responsibilities in preparing any document for any use.

4. A present project is being carried out by me, financially supported primarily by Hyal Pharmaceutical Sweden AB, a related company to Hyal Pharmaceutical Corporation, the Assignee of U.S. Application Serial No. 07/657,908. My research is also supported by King Gustaf V:s 80-year fund, the Department of Medical and Physiological Chemistry, University of Uppsala and Pharmacia and Upjohn.

5. Prior to 1989, I was aware of what was transpiring in the technical areas of uses of hyaluronic acid. Prior to my first contact with Hyal Pharmaceutical Corporation, I had experience from experimental *in vitro* as well as *in vivo* work with hyaluronic acid (HA) and other polysaccharides and macro molecules in different biological systems. From 1986 (after my doctoral thesis), my work has focused on HA metabolism and HA receptors. I have worked with respect to these issues, not only at the University of Uppsala but also for Pharmacia (Pharmacia Ophthalmics) as a Consultant, then, mainly for the use of HA in the eye. I have supervised graduate students on HA metabolism since 1987. I have written a substantial number of articles which relate specifically to hyaluronic acid and these are identified as numbers, 14, 15, 19, 21, 22, 23-38 (inclusive), 40, 41, 48 (written in 1989), and 51-62 inclusive in my Curriculum Vitae.

6. After I heard about the invention of Drs. Falk and Asculai relating to the delivery of drugs and medicines and therapeutic agents by the use of hyaluronic acid to a site of disease or condition in need of treatment, I changed my thinking with respect to hyaluronic acid (HA) as a drug delivery system. Before hearing about the active transport and delivery by HA of the medicines and therapeutic agents developed by Drs. Falk and Asculai, I did not think that drug delivery was possible using HA to a cancerous tumour or to a site of inflammation. I knew about the metabolism of HA by the liver and the passage of the HA into the lymph nodes but did not believe such drug delivery was possible.

7. I have carefully reviewed International Application No. PCT/CA 90/00306 published under International Publication No. WO 91/04058 which I am advised by Ivor Hughes, Counsel for Hyal Pharmaceutical Corporation and, do verily believe was filed in the U.S. Patent Office as the National Entry Application in the U.S. Patent Office under Serial No. 07/675,908 and have determined that the invention disclosed therein is the invention to which I am referring in paragraph 6 of this my Declaration. Particularly, I am referring to the invention relating to the use of dosage amounts of hyaluronic acid between about 10 mg. and 1,000 mg./70 kg. person of the amount of hyaluronic acid and salts thereof with optimal doses tending to range between 50 and 350 mg./70 kg. individual and doses which may exceed 3,000 mg./70 kg. individual without any adverse effects, together with an effective dosage amount of a medicine which would be used to treat the disease or condition (discussed at p. 26, lines 32-37 of International Published Application WO 91/04058). By the use of the dosage amounts containing the HA and drug/medicine, methods of treatment of the disease or condition have been developed by Drs. Falk and Asculai which alter the drugs' and medicines' performance in the human body.

8. That by providing the dosage amounts to cancer patients who had been unresponsive to conventional treatment (which patients I consider terminal), patients' quality of life and longevity increased according to the examples in the case. In some cases, patients who appear to have been terminally ill went into remission and were still alive at the date of the Application. Thus, after hearing of Drs. Falk and Asculai's invention and having observed the data in animals, my thoughts changed and I look for explanations. As time has passed, I have determined that accessible receptors can be expressed at tumours (the tumour being the site of the disease or condition) and when the hyaluronic acid carries a cytostatic/cytotoxic drug, the hyaluronic acid takes the drug to the tumour and

other sites of hyaluronic acid uptake. This would then increase the efficacy of the drug on tumour elimination. As a result of the teachings in the Application, I became very excited and concluded that the said invention will have a real impact with respect to the treatment of disease and conditions in patients particularly suffering from cancer and inflammation, two technical areas where I previously believed the combination would not work.

9 When examining International Publication No. WO 91/04058, I was asked to comment thereon and particularly, the teachings in the Application that would enable persons skilled in the art to understand the teachings in the Application and be able to reproduce same. While I am a Master of Pharmacy and a Doctor of Medical Science, and have been Associate Professor since 1991 in the Medical and Physiological Chemistry and have written a substantial number of articles with respect to hyaluronan, I am not a practising physician but, do have a good understanding with respect to the use of hyaluronic acid (HA) and in fact, have filed patent applications in respect of the uses of HA in the U.S. Patent Office in respect of methods of treatment employing HA.

10. I was asked whether or not pharmaceutically acceptable salts or non-toxic salts were taught by International Publication No. WO 91/04058. It is quite clear to me that pharmaceutically acceptable salts are implicitly meant to be used in the dosages. As stated on page 1, lines 4-5 by the expression "formulations suitable for use to treat conditions and disease", persons skilled in the art would understand that salts beneficial to the patients are to be used and not those that would cause toxicity to the patient.

11. With respect to the molecular weights of HA to be used, the application refers to batches that have worked well according to Drs. Falk and Asculai, e.g. from Sterivet, the molecular weight range was 150,000 to 250,000 daltons and

from Lifecore, less than 750,000 daltons. Persons skilled in the art would, therefore, use hyaluronic acid with a mean molecular weight of between 150,000 daltons to 750,000 daltons. They would know to make the dosages such that the amount and viscosity do not cause toxicity of hyaluronic acid. Toxicity of hyaluronic acid would be due to viscosity problems from very high amounts of circulating hyaluronic acid. There are cases with Wilms' tumour where it is described that the viscosity of the blood is increased and also associated with clotting disorders. The problem would be limited to patients with Wilms' tumour, with Werner's syndrome, or with a dramatically decreased clearance capacity such as in advanced liver disease.

12. Calcium channel blockers are taught in International Publication No. WO 91/04058 at page 35, line 22 and one example, nifedipine is taught at page 35, line 23 under sub-paragraph 16.

13. With respect to the teachings of the prior art, I am satisfied that with respect to the teachings of U.S. Patent 4,736,024, there are no teachings of the active delivery or transport of medicine and/or therapeutic agents to a site in need of treatment by the hyaluronic acid.

14. Della Valle, U.S. Patent 4,736,024 exemplifies what was known in the industry in about 1987 in respect of topically applied formulations containing HA and substances carried by the HA when applied to the eye for absorption of the substance carried in the HA by the eye. It was my opinion that the said formulations, applied topically, cause the HA to adhere to the eye and permit the substance carried by the HA to leach or leak out therefrom and be absorbed by the local area to which HA contacts. This is to what precisely the teachings of Della Valle, U.S. Patent 4,736,024 relate.

15. Della Valle provides combinations of HA and medicine but, does not provide appropriate dosages to achieve the active delivery and transport of the medicine as is taught by International Publication No. WO 91/04058. While Della Valle discusses the use of the formulations for ophthalmics (the eye) and the patent suggests that the formulations can be used for dermatology and, at column 4, that it is possible to conclude by analogy that the medicines can be used in other fields such as otorhinolarynology, odontology, or in internal medicine for example, endocrinology where it is possible to effect treatments with preparations for intradermic absorption or absorption through the mucous for example, rectal or intranasal absorption, for example, nasal sprays, or inhalations in the oral cavity and in the pharynx. In my opinion, the nature of the formulations does not change. It is expected that the formulations will, once again, stick to the surface to which they have been applied and the medicines will leach from. It is clear to me that the retard effect of the HA and leaching effect of the medicine from the HA is only substantiated for the special case of the cornea and only by analogy does Della Valle suggest the statements apply to topical formulations applied elsewhere topically.

16. I have arrived at this conclusion based on the teachings of the patent as a whole and refer the Examiner's attention to column 1, line 46:

> "When the medicaments are administered in the form of concentrated solutions with the elastic-viscose characteristics or in solid form, it is possible to obtain films on the corneal epithelium which are homogeneous, stable, perfectly transparent and which adhere well guaranteeing prolonged bioavailability of the drug thereby forming excellent preparations with a retard effect."

17. A similar statement is made at column 2, lines 41-51:

18. It is therefore clear to me and would be to persons skilled in the art that the better bioavailability of the active substance which is provided by the use of the hyaluronic acid is provided by a film on the surface of the eye which adheres well and which provides a preparation with a retard effect. In other words, there is no active delivery but rather a leaching from the form of hyaluronic acid - nothing more.

19. Such a formulation may be used with the fields discussed at the bottom of column 4, top of column 5, namely, the topical application and the leaching therefrom. There is, however, no targeting or transport suggested.

20. It is therefore clear to me that while combinations of hyaluronic acid with medicine have been taught to a limited extent, the formulations are for purposes and effects which do not permit transportation of the medicine by the hyaluronic acid to the site in need of treatment.

21. As our knowledge has evolved, we have now discovered that when HA is given systemically to the body for example, intravenously or by injection, the liver and other parts of the body including the sites in need of treatment, unfilled receptors for hyaluronic acid became filled. As the liver has the largest capacity to take up hyaluronic acid, this organ's receptors have to be filled or blocked, in order to provide the sites in need of treatment with drugs carried by hyaluronic acid. This could be achieved by a high dose of hyaluronic acid or other agents that interact with the hyaluronic acid liver receptors. If minimum amounts are not given which satisfy the liver, then nothing will reach the site of the disease or condition in need of treatment (generally, normal tissue has filled receptors or very minimal numbers of unfilled receptors). Thus, it is important with respect to the minimum amounts of hyaluronic acid.

22. There is also no teaching of the use of hyaluronic acid in combination with other drugs by Schultz, U.S. Patent 4,808,576. Schultz does not teach that when HA is applied topically that it is transported to a remote site and for example, reduces inflammation. In order to transport HA administered topically in Schultz, Schultz requires the use of a compatible transdermal carrier. While any recognized carrier will do, according to Schultz, DMSO is preferred (see column 6, lines 1-9). Thus, the hyaluronic acid applied topically is carried by the DMSO into the body (all over the body) by the bodily functions and by the effects of the DMSO in the body and some of it arrives at the site for which treatment is desired. HA does not work topically by itself (see column 12, lines 14-17). While other transdermal carriers are proposed, they themselves transport the HA, not the HA itself. The HA is the therapeutic agent, if anything.

23. In my opinion, U.S. Patent 4,808,576 and the teachings thereof is not relevant to procedures that use HA as the vehicle for another therapeutic agent or as a means to deliver other therapeutic substances to a desired site of action. Throughout the document, it is made clear that hyaluronan itself is the therapeutic agent. It is stated that the topical application of HA requires a compatible and recognized transdermal carrier and sites methyl or sodium salicylate, benzyl alcohol, oleic acid (amounts of these agents are not specified) and others, particularly DMSO.

24. I have also been asked to review the teachings of Seifter U.K. Patent 769,287 specifically as to what Seifter does and does not teach.

25. Seifter's claim is that partially depolymerized HA allows substances to spread more rapidly in the tissues and also that these low molecular weight oligosaccharides have a lipemia clearing effect when given intravenously, orally,

or subcutaneously. It is clear to me that the invention is limited to depolymerized hyaluronic acid as it is described on page 2, line 15 that the hyaluronic acid must be treated by enzymatic cleavage "for at least five minutes". It is clear that undepolymerized hyaluronic acid used in International Published Application WO 91/04058 is not taught by Seifter, U.K. Patent 769,287. The latter patent would not, in my opinion, direct anyone skilled in the art to conclude that undepolymerized hyaluronic acid could be used as a transport agent.

26. In summary, it is therefore clear to me that the combinations presented in International Published Application No. WO 91/04058 are new, useful, and inventive as are the methods of treatment using the dosages. None of the references provided to me taught me the said invention, nor does any combination of these references teach the dosage amounts or the methods of treatment. In fact, there is no recognition in any patents or prior art of which I am aware which teaches the invention. Hence, my surprise when I first learned of the invention that it was possible to deliver by the use of HA a medicine or therapeutic agent to a tumour or a site of inflammation. This was completely contrary to my understanding of what was known with respect to hyaluronic acid in or about 1989.

27. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements will jeopardize the validity of the application and any patent issuing thereon.

EXECUTED this 29th day of August, 1996.

STEFAN GUSTAFSON

EXHIBIT 1

Curriculum vitae

Stefan Gustafson

Date of Birth: March 18 1956
Marital status: Married
Number of children: Two; Johan and Jenny

Bachelor of Pharmacy 1978
Master of Pharmacy 1980
Honors degree in Medical and Physiological Chemistry 1980
Teaching assistent at the Dept of Medical and Physiological Chemistry 1980
Lecturer in biochemistry 1980-1982
Post graduate scholarship recipient 1981-1986
Research on lipoprotein metabolism at Dept of Medicine,
Div of metabolism, Univ of California, San Diego March-Oct 1982
Doctor of Medical Science, Univ of Uppsala 1986
Post doc work on Hyaluronan metabolism in Torvard
C. Laurents lab 1986-1989
Lecturer in Medical and Physiological chemistry 1986-1993
Research assistent position at Dept of Med&Phys Chemistry 1989
Associate professor in Medical and Physiological Chemistry 1991
Senior scientist position at Hyal Pharmaceutical Sweden 1993

I have mainly been studying metabolism of lipoproteins and hyaluronan, especially receptor mediated endocytosis in reticuloendothelial cells (ie. macrophages and liver endothelial cells). The work consists of purification and characterization of lipoproteins, polysaccharides and cell surface receptors for these ligands, together with binding, uptake and clearance studies *in vitro* and *in vivo*. I have also developed immunological assays for proteins in serum and lymph, and used these in clinically oriented projects. The majority of the work is carried out at the Dept. of Medical and Physiological Chemistry, Biomedical Centre, Uppsala University. I also have collaboration with Pharmacia & Upjohn, Depts. of Surgery, Dermatology and Oncology at the University hospital (primarily Wilhelm Graf, Lennart Juhlin, Jan-Erik Westlin and Hans Hagberg), the PET-centre at University of Uppsala, and other departments at the Biomedical Centre and the University hospital.
I supervise several graduate students (one became doctor of Medical Science May 1996) and medical students working primarily with hyaluronan receptor studies.

My project is supported primarily by Hyal Pharmaceutical Sweden AB, but also by King Gustaf V:s 80-year fund, the Department of Medical and Physiological Chemistry, University of Uppsala and Pharmacia and Upjohn.

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